

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 39/39</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 95/17209</b> <b>(43) International Publication Date:</b> 29 June 1995 (29.06.95)
<b>(21) International Application Number:</b> PCT/EP94/04227 <b>(22) International Filing Date:</b> 15 December 1994 (15.12.94) <b>(30) Priority Data:</b> 9326253.3                      23 December 1993 (23.12.93)      GB <b>(71) Applicant (for all designated States except US):</b> SMITHKLINE BEECHAM BIOLOGICALS (S.A.) [BE/BE]; 89, rue de l'Institut, B-1330 Rixensart (BE). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> MOMIN, Patricia, Marie [BE/BE]; SmithKline Beecham Biologicals (S.A.), 89, rue de l'Institut, B-1330 Rixensart (BE). GARCON, Nathalie, Marie-Josephe [FR/FR]; SmithKline Beecham Biologicals (S.A.), 89, rue de l'Institut, B-1330 Rixensart (BE). <b>(74) Agent:</b> DALTON, Marcus, Jonathan, William; SmithKline Beecham, Corporate Intellectual Property, SB House, Great West Road, Brentford, Middlesex TW8 9BD (GB).		<b>(81) Designated States:</b> AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> VACCINES  <b>(57) Abstract</b>  The present invention provides vaccine compositions comprising an oil in water emulsion optionally with 3 De-O-acylated monophosphoryl lipid A and QS21. The vaccines' compositions are potent inducers of a range of immune responses.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

- 1 -

### Vaccines

The present invention relates to novel vaccine formulations, to methods of their production and to their use in medicine. In particular, the present invention relates to an oil in water emulsion. Such emulsions comprise tocopherol, squalene, Tween 80, Span 85 and Lecithin and have useful adjuvant properties. Vaccines containing QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina, and/or 3 De-O-acylated monophosphoryl lipid A (3 D-MPL), together with such oil in water emulsions also form part of the invention.

3 De-O-acylated monophosphoryl lipid A is known from GB2220 211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in International Patent Application No. 92/116556.

QS21 is a Hplc purified non toxic fraction of a saponin from the bark of the South American tree Quillaja Saponaria Molina and its method of its production is disclosed (as QA21) in US patent No. 5,057,540.

Oil in water emulsions per se are known in the art, and have been suggested to be useful as adjuvant compositions (EPO 399843).

The present invention is based on the surprising discovery that an oil in water emulsion of the present invention, which unlike emulsions of the prior art contain tocopherol, as such or in combination with QS21 and/or 3 D-MPL enhance immune responses to a given antigen. Such enhancement available affords better immunological responses than hitherto before.

Additionally the oil in water emulsions of the present invention when formulated with 3 D-MPL and QS21 are preferential stimulators of IgG2a production and TH1 cell response. This is advantageous, because of the known implication of TH1 response in cell mediated response. Indeed in mice induction of IgG2a is correlated with such an immune response.

For example a vaccine formulation of the HIV antigen gp120 in such a combination results in a powerful synergistic induction of gp120 protein specific immune responses.

- 2 -

The observation that it is possible to induce strong cytolytic T lymphocyte responses is significant as these responses, in certain animal models have been shown to induce protection against disease.

5

The present inventors have shown that the combination of the adjuvants QS21 and 3D-MPL together with an oil in water emulsion with an antigen results in a powerful induction of CS protein specific CTL in the spleen. QS21 also enhances induction of CTL on its own, while 3D-MPL does not.

10

Induction of CTL is easily seen when the target antigen is synthesised intracellularly (e.g. in infections by viruses, intracellular bacteria, or in tumours), because peptides generated by proteolytic breakdown of the antigen can enter the appropriate processing pathway, leading to presentation in association with class I molecules on the cell membrane. However, in general, pre-formed soluble antigen does not reach

15

this processing and presentation pathway, and does not elicit class I restricted CTL. Therefore conventional non-living vaccines, while eliciting antibody and T helper responses, do not generally induce CTL mediated Immunity. The combination of the two adjuvants QS21 and 3D-MPL together with an oil in water emulsion can

20

overcome this serious limitation of vaccines based on recombinant proteins, and induce a wider spectrum of immune responses.

CTL specific for CS protein have been shown to protect from malaria in mouse model systems (Romero et al. Nature 341:323 (1989)). In human trials where volunteers were immunised using irradiated sporozoites of *P. falciparum*, and shown to be protected against subsequent malaria challenge, induction of CTL specific for CS epitopes was demonstrated (Malik et al. Proc. Natl. Acad. Sci. USA 88:3300 (1991)).

25

The ability to induce CTL specific for an antigen administered as a recombinant molecules is relevant to malaria vaccine development, since the use of irradiated sporozoites would be impractical, on the grounds of production and the nature of the immune response.

30

RTS is a hybrid protein comprising substantially all the C-terminal portion of the circumsporozoite (CS) protein of *P. falciparum* linked via four amino acids of the preS<sub>2</sub> portion of Hepatitis B surface antigen to the surface (S) antigen of hepatitis B virus. Its full structure is disclosed in co-pending International Patent Application No. PCT/EP92/02591, published under Number WO 93/10152 claiming priority from UK

35

patent application No.9124390.7. When expressed in yeast RTS is produced as a lipoprotein particle, and when it is co-expressed with the S antigen from HBV it produces a mixed particle known as RTS,S.

5 In addition to human immunodeficiency virus and malaria vaccines, the ability to induce CTL responses would benefit vaccines against herpes simplex virus, cytomegalovirus, and generally all cases where the pathogen has an intracellular life stage.

10 Likewise, CTL specific for known tumour antigens could be induced by a combination of a recombinant tumour antigen and the two adjuvants. This would allow the development of anti cancer vaccines.

In certain systems, the combination of 3D-MPL and QS21 together with an oil in  
15 water emulsion have been able to synergistically enhance interferon  $\gamma$  production. The present inventors have demonstrated the potential of 3D-MPL and QS21 together with an oil in water emulsion by utilising a herpes simplex antigen known as gD<sub>2t</sub>. gD<sub>2t</sub> is a soluble truncated glycoprotein D from HSV-2 and is produced in CHO cells according to the methodology Berman et al. Science 222 524-527.

20 IFN- $\gamma$  secretion is associated with protective responses against intracellular pathogens, including parasites, bacteria and viruses. Activation of macrophages by IFN- $\gamma$  enhances intracellular killing of microbes and increases expression of Fc receptors. Direct cytotoxicity may also occur, especially in synergism with lymphotoxin (another  
25 product of TH1 cells). IFN- $\gamma$  is also both an inducer and a product of NK cells, which are major innate effectors of protection. TH1 type responses, either through IFN- $\gamma$  or other mechanisms, provide preferential help for IgG2a immunoglobulin isotypes.

Glycoprotein D is located on the viral envelope, and is also found in the cytoplasm of  
30 infected cells (Eisenberg R.J. et al. J. of Virol. 1980 35 428-435). It comprises 393 amino acids including a signal peptide and has a molecular weight of approximately 60kD. Of all the HSV envelope glycoproteins this is probably the best characterized (Cohen et al. J. Virology 60 157-166). In vivo it is known to play a central role in viral attachment to cell membranes. Moreover, glycoprotein D has been shown to be  
35 able to elicit neutralizing antibodies in vivo (Eing et al. J. Med Virology 127: 59-65). However, latent HSV2 virus can still be reactivated and induce recurrence of the disease despite the presence of high neutralizing antibodies titre in the patients sera. It

- 4 -

is therefore apparent that the ability to induce neutralizing antibody alone is insufficient to adequately control the disease.

5 In order to prevent recurrence of the disease, any vaccine will need to stimulate not only neutralizing antibody, but also cellular immunity mediated through T-cells, particularly cytotoxic T-cells.

10 In this instance the gD<sub>2t</sub> is HSV2 glycoprotein D of 308 amino acids which comprises amino acids 1 through 306 of the naturally occurring glycoprotein with the addition of Asparagine and Glutamine at the C terminal end of the truncated protein. This form of the protein includes the signal peptide which is cleaved to yield a mature 283 amino acid protein. The production of such a protein in Chinese Hamster ovary cells has been described in Genentech's European patent EP-B-139 417.

15 The mature truncated glycoprotein D (rgD<sub>2t</sub>) or equivalent proteins secreted from mammalian cells, is preferably used in the vaccine formulations of the present invention.

20 The formulations of the present invention are very effective in inducing protective immunity in a genital herpes model in guinea pigs. Even with very low doses of antigen (e.g. as low as 5 µg rgD<sub>2t</sub>) the formulations protect guinea pigs against primary infection and also stimulate specific neutralising antibody responses. The inventors, utilising formulation of the present invention, have also demonstrated Effector cell mediated responses of the TH1 type in mice.

25 Accordingly, in one preferred embodiment of the present invention provides a vaccine or pharmaceutical formulation comprising an antigen in conjunction with 3 De-O-acylated monophosphoryl lipid A, QS21 and an oil in water emulsion wherein the oil in water emulsion comprises a metabolisable oil, such as squalene, alpha tocopherol and  
30 tween 80. Such a formulation is suitable for a broad range of monovalent or polyvalent vaccines. Additionally the oil in water emulsion may contain span 85. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in International patent application published under No. 92116556 - SmithKline Beecham Biologicals  
s.a.

35 The oil in water emulsion may be utilised on its own or with other adjuvants or immuno-stimulants and therefore an important embodiment of the invention is an oil in

water formulation comprising squalene or another metabolisable oil, alpha tocopherol, and tween 80. The oil in water emulsion may also contain span 85 and/or Lecithin.

Preferably the vaccine formulations will contain an antigen or antigenic composition  
5 capable of eliciting an immune response against a human or animal pathogen, which antigen or antigenic composition is derived from HIV-1, (such as gp120 or gp160), any of Feline Immunodeficiency virus, human or animal herpes viruses, such as gD or derivatives thereof or Immediate Early protein such as ICP27 from HSV1 or HSV2, cytomegalovirus ((esp Human)(such as gB or derivatives thereof), Varicella Zoster  
10 Virus (such as gpI, II or III), or from a hepatitis virus such as hepatitis B virus for example Hepatitis B Surface antigen or a derivative thereof, hepatitis A virus, hepatitis C virus and hepatitis E virus, or from other viral pathogens, such as Respiratory Syncytial virus, human papilloma virus or Influenza virus, or derived from bacterial pathogens such as Salmonella, Neisseria, Borrelia (for example OspA or OspB or  
15 derivatives thereof), or Chlamydia, or Bordetella for example P.69, PT and FHA, or derived from parasites such as plasmodium or Toxoplasma.

The formulations may also contain an anti-tumour antigen and be useful for immunotherapeutically treating cancers.

20

In an immunotherapeutic animal model for B cell lymphoma, where BCL-1 mouse lymphoma cells are administered intraperitoneally to Balb/c mice on day 0, and mice are vaccinated on days 3, 10 and 20 with the BCL-1 Idiotypic formulation SB62/MPL/QS21 stands out as the most potent, both with respect to antibody titers,  
25 and with respect to survival (the only group with 100% survival). Similarly the ability of this formulation to stimulate cytotoxic T lymphocytes to the antigens included make them a good candidate for formulation of cancer antigens (eg melanoma antigens MAGE-1 and MAGE-3 for immunotherapy of tumors by active vaccination).

30 The formulation may also be useful for utilising with herpetic light particles such as described in International Patent Application No. PCT/GB92/00824 and, International Patent Application No. PCT/GB92/00179.

Derivatives of Hepatitis B Surface antigen are well known in the art and include, inter  
35 alia, those PreS<sub>1</sub>, PreS<sub>2</sub> S antigens set forth described in European Patent applications EP-A-414 374; EP-A-0304 578, and EP 198-474. In one preferred aspect the vaccine formulation of the invention comprises the HIV-1 antigen, gp120, especially when

- 6 -

expressed in CHO cells. In a further embodiment, the vaccine formulation of the invention comprises gD<sub>2</sub>t as hereinabove defined.

5 In a further aspect of the present invention there is provided a vaccine as herein described for use in medicine.

The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1; preferably 1 : 5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is 2.5:1 to 1:1 3D MPL: QS21. Typically for human administration QS21 and  
10 3D MPL will be present in a vaccine in the range 1 µg - 100 µg, preferably 10 µg - 50 µg per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha tocopherol is equal or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be  
15 advantageous that the vaccines of the present invention will further contain a stabiliser.

Vaccine preparation is generally described in New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A.  
20 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for example, by Likhite, U.S. Patent 4,372,945 and by Armor et al., U.S. Patent 4,474,757.

25 The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 µg of protein, preferably 2-100 µg, most preferably 4-40 µg. An  
30 optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects may receive one or several booster immunisation adequately spaced.

35 The formulations of the present invention maybe used for both prophylatic and therapeutic purposes.

Accordingly in one aspect, the invention provides a method of treatment comprising administering an effective amount of a vaccine of the present invention to a patient.

The following examples illustrate the invention.

5

### Examples

**Example 1** Vaccine formulation comprising the gp120 antigen of HIV-1.

- 10 The two adjuvant formulations were made each comprising the following oil in water emulsion component.

SB26: 5% squalene 5% tocopherol 0.4% tween 80; the particle size was 500 nm size

SB62: 5% Squalene 5% tocopherol 2.0% tween 80; the particle size was 180 nm

15

1(a) Preparation of emulsion SB62 (2 fold concentrate)

- 20 Tween 80 is dissolved in phosphate buffered saline (PBS) to give a 2% solution in the PBS. To provide 100 ml two fold concentrate emulsion 5g of DL alpha tocopherol and 5ml of squalene are vortexed to mix thoroughly. 90ml of PBS/Tween solution is added and mixed thoroughly. The resulting emulsion is then passed through a syringe and finally microfluidised by using an M1105 microfluidics machine. The resulting oil droplets have a size of approximately 180 nm.

- 25 1(b) Preparation of emulsion SB26

This emulsion was prepared in an analogous manner utilising 0.4% tween 80.

- 30 1(c) Other emulsions as depicted in Table 1 were made in an analogous manner. These are tested in the experiments as detailed in the following examples.

1(d) Preparation of gp 120 QS21/3D MPL oil in water formulation.

- 35 To the emulsion of 1 a) or b) or c) an equal volume of twice concentrated rgp120 (either 20µg or 100µg) was added and mixed. This was combined with 50µg/ml of 3D-MPL and 20µg/ml of QS21 to give the final formulation. Buffer was sed according to salt content and pH.

Table 3 shows the effectiveness of SB26, utilising gp120 from HIV and 50µg/ml 3D MPL (MPL) and 20µg/ml of QS21. The results show the geometric mean titre (GMT) after the second (P11) and third (P111) inoculations as well as cell mediated responses (CMI) to lymphocyte proliferation and  $\gamma$  interferon production.

5

## Example 2

Introduction: Evaluation of an HIV gp 120 emulsion system

- 10 In this experiment, four emulsions are compared [SB26, SB 62, SB40, SB61]. The influence of each formulation's component (antigen, emulsion, 3D- MPL, QS21) is evaluated.

### 2(b) Groups of animals utilised

15

There are 22 groups of 5 animals each group received a different vaccine formulation.

- gr 1-4: gp 120 (10µg) / no emuls ± [3D-MPL, QS21]
- gr 5-9: gp 120 (10µg) / SB26 ± [3D-MPL, QS21]
- 20 - gr 10: no antigen / SB26 + [3D-MPL, QS21]
- gr 11-12: gp 120 (10µg) / SB62 ± [3D-MPL, QS21]
- gr 13-16: gp 120 (10µg) / SB40 ± [3D-MPL, QS21]
- gr 17-20: gp 120 (10µg) / SB61 ± [3D-MPL, QS21]
- gr 21-22: gp 120 (5µg) / SB26 ± [3D-MPL, QS21]

25

- Assays: - antibody titers to gp 120W61D and isotype analysis (all groups)

### 2(c) Immunization and bleeding schedule

- 30 - animals were immunized with gp 120W61D, formulated in different o/w emulsions in the presence of 5µg 3D-MPL and 5µg QS21 per dose. Negative controls received the equivalent formulations without any antigen.
- animals were immunized subcutaneously at day 0 and 14. Each injection dose
- 35 was administered in a 100µl volume.
- blood samples were obtained before Immunization (day 0) and after Immunization on days 14 (post I), 21 and 28 (7 and 14d. post II).

**2(d) Analysis of the serological response:**

- the 14 days post I and post II serological response was evaluated in a direct ELISA assay to gp 120W61D.
- the 14 days post II response was also characterized regarding the isotypes of gp 120W61D specific antibodies induced in mice after immunization.

**3 RESULTS AND DISCUSSION:**

The results are depicted on Table 2

**a) Comparison of emulsions in the presence or absence of 3D-MPL/QS21:**

15

- Addition of emulsions SB26, SB40 or SB62 to the antigen induces higher antibody titers; In the absence of immunostimulants, the gp 120 specific antibodies are essentially IgG1.

20

- Addition of immunostimulants 3D-MPL and QS21 induces a huge serological response and a shift of antibodies from IgG1 type to IgG2a/IgG2b. This correlated with cell mediated immunity.

The preferred combination is [SB26 + MPL + QS21].

25

**c) gp120/SB26 formulation:**

No significant difference in serological response is observed between group 8 and group 9: addition of the gp 120 before or after the other components of the formulation.

30

**d) Antigen dose:**

Both 5 and 10 µg of gp 120 formulated in SB26 induce high serological response (groups 5-8 and 21-22).

**35 Example 3 HSV rgD<sub>2</sub>t formulation**

In analogous manner to that set forth in Example 1a) formulation comprising the herpes simplex antigen rgD<sub>2</sub>t was made and used to vaccinate guinea pigs. Such

formulation induced protection against both recurrent and initial disease in the guinea pig model.

#### Example 4

#### 5 Screening of adjuvants for induction of protective anti lymphoma responses using idiotype as immunogen.

Therapeutic vaccination of Balb/c mice with idiotype from BCL1 lymphoma cells.

- 10 A review of the BALB/C B-cell lymphoma model is discussed by Yefenoh et al. Current opinions Immunobiology 1993 5:740-744.

- Groups of 10 mice are injected (ip) with  $10^4$  tumor cells at day 0, and vaccinated with 100 µg of KLH- coupled immunoglobulin directed against BCL 1 epitoped (ratio of  
15 KLH/Ig: 1/1), in different adjuvant formulations at days 3, 10, 20 (sc immunization in the back). Level of serum antibodies to KLH and to idiotype, as well as mouse death are monitored.

Formulations tested:

group#	adjuvant	MPL: 10µg QS21: 10µg
1	none (no antigen)	
2	none	
3	Freund	
4	Alum	
5	Alum/MPL	
6	Alum/MPL/QS21	
7	QS21	
8	MPL/QS21	
9	SB62MPL	
10	SB62/MPL/QS21	

- 20 groups 12-15: different adjuvants without antigen

- Formulations 8, 9, 10, behaved consisently better as compared to the others.  
Formulation 10 stands out as the most potent, both with respect to antibody titers, and  
25 with respect to survival (the only group with 100% survival).

**EXAMPLE 5 Various formulations of RTS,S****a) Evaluated in monkeys**

5

RTS,S is described in International patent application no. WO93/10152 and was formulated for vaccination of Rhesus monkeys. Five animals were in each group:

- |    |           |  |
|----|-----------|--|
| 10 | Group I   | RTS,S, 3D-MPL(50 $\mu$ ), AL(OH) <sub>3</sub>      |
|    | Group II  | RTS,S, QS21(20 $\mu$ ), AL(OH) <sub>3</sub>        |
|    | Group III | RTS,S, 3D-MPL(50 $\mu$ ), QS21(20 $\mu$ )          |
|    | Group IV  | RTS,S, 3D-MPL(50 $\mu$ ), QS21 AL(OH) <sub>3</sub> |
|    | Group V   | RTS,S, 3D-MPL(10 $\mu$ ), QS21 AL(OH) <sub>3</sub> |
|    | Group VI  | RTS,S, 3D-MPL(50 $\mu$ ), QS21 SB60                |

15

The animals were inoculated and bled at 14 days post first immunisation and 12 days post second immunisation and tested for Anti hepatitis B surface antigen immunoglobulin. As can be seen from figure 1, animals receiving RTS,S, in SB60 had antibody titres almost six fold higher than any other group.

20

**b) Various formulations of RTS,S - Evaluated in mice**

7 groups of animals received the following formulations

- |    |         |                                  |
|----|---------|----------------------------------|
| 25 | Group 1 | RTS,S SB62                       |
|    | Group 2 | RTS,S QS21 3D-MPL                |
|    | Group 3 | RTS,S QS21 3D-MPL SB26           |
|    | Group 4 | RTS,S 3D-MPL A1(OH) <sub>3</sub> |
|    | Group 5 | RTS,S A1(OH) <sub>3</sub>        |
| 30 | Group 6 | Plain                            |
|    | Group 7 | Negative control                 |

(RTS,S - 5 $\mu$ g/dose, 3 D-MPL 5 $\mu$ g/dose QS21 5 $\mu$ g/dose)

- 35 The animals were inoculated and bled at 15 days post first immunisation and at day 7 and 15 post second immunisation and assayed for anti HBSAg antibody subtype. As can be seen from figure 2, the emulsion SB62 when formulated with QS21 and 3D-

MPL enhances preferentially and in a synergistic fashion the IgG2a antibody response while SB 62 alone or 3 D- MPL / QS21 induce a poor IgG2a response.

#### EXAMPLE 6: Evaluation of different B burgdorferi OspA formulations

5

##### 6.1 Evaluation of different formulations of B burgdorferi ZS7 Osp A lipoproteins.

OspA lipoprotein for B burgdorferi is described in European Patent Application 0418 827 Max Plank et al.

10

The following formulations were tested in balb/c mice

1. OspA + A1(OH)<sub>3</sub>
2. OspA + A1(OH)<sub>3</sub> + 3D-MPL (10 $\mu$ )
- 15 3. OspA + A1(OH)<sub>3</sub> + 3D-MPL (30 $\mu$ )
4. OspA + A1(OH)<sub>3</sub> + 3D-MPL (10 $\mu$ ) + QS21 (5 $\mu$ )
5. OspA + A1(OH)<sub>3</sub> + 3D-MPL (30 $\mu$ ) + QS21 (15 $\mu$ )
6. OspA + SB60 + 3D-MPL (10 $\mu$ ) + QS21 (5 $\mu$ )
7. OspA + SB60 + 3D-MPL (30 $\mu$ ) + QS21 (15 $\mu$ )

20

and antibody titres and sub types studied seven days following a first inoculation and seven days post second inoculation (inoculations were at day 0, and 14).

The results depicted graphically in figures 3 and 4 and show that the formulations of the present invention induce high levels of antibodies and these are preferentially of the IgG2a subtype.

25

#### EXAMPLE 7:

##### 30 a). HSV-2 ICP 27

Female Balb/c mice were immunized on day 0 and day 14 in the hind foot-pads with various formulations of NS1-ICP27. Each injection contained 5  $\mu$ g of NS1-ICP27 and combinations of SB26 oil-in-water emulsion, QS21 (10  $\mu$ g) and MPL (25  $\mu$ g).

35

Popliteal lymphnode cells were obtained on day 28 and stimulated in vitro with syngeneic P815 cells transfected with the ICP27 gene. The cultures were then tested for specific cytolytic activity on P815 target cells transfected with ICP27 and P815 ICP27 negative controls.

- 13 -

Specific lysis results at different effector:target (E:T) ratios for different immunization groups were as follows:

ICP 27 (5 $\mu$ g)

5	E:T	P815	P815 transfected with ICP 27 clone 121
	100:1	-1	0
	30:1	-2	-3
	10:1	3	0
	3:1	1	0
10	1:1	2	2
	0.3:1	2	2

ICP 27 (5 $\mu$ g) + MPL (25 $\mu$ g)

	E:T	P815	P815 transfected with ICP 27 clone 121
15	100:1	5	7
	30:1	2	2
	10:1	1	2
	3:1	-1	-1
	1:1	-2	-2
20	0.3:1	-4	-1

ICP 27 (5 $\mu$ g) + QS21 (10 $\mu$ g)

	E:T	P815	P815 transfected with ICP 27 clone 121
	100:1	4	17
25	30:1	5	10
	10:1	3	7
	3:1	4	5
	1:1	3	5
	0.3:1	0	1
30			

ICP 27 (5 $\mu$ g) + SB26

	E:T	P815	P815 transfected with ICP 27 clone 121
	100:1	5	20
	30:1	1	19
35	10:1	2	12
	3:1	-2	7
	1:1	1	5
	0.3:1	1	2

- 14 -

ICP 27 (5µg) + MPL (25µg) + QS21 (10µg)

E:T P815 P815 transfected with ICP 27 clone 121

100:1 4 13  
 5 30:1 5 12  
 10:1 4 17  
 3:1 1 3  
 1:1 0 3  
 0.3:1 -1 -2

10

ICP 27 (5µg) + MPL (25µg) + QS21 (10µg) + SB26

E:T P815 P815 transfectés avec ICP27 clone 121

100:1 2 20  
 30:1 0 17  
 15 10:1 3 19  
 3:1 3 8  
 1:1 1 6  
 0.3:1 2 3

20 Low ICP27 specific % lysis was obtained in immunization groups:

ICP 27 (5µg) + QS21 (10µg)

ICP 27 (5µg) + SB26

ICP 27 (5µg) + MPL (25µg) + QS21 (10µg)

25 ICP 27 (5µg) + MPL (25µg) + QS21 (10µg) + SB26

while

ICP 27 (5µg)

30 ICP 27 (5µg) + MPL (25µg)

were negative.

Thus these data show induction of CTL by recombinant NS1-ICP27 in  
 oil-in-water emulsion alone or with QS21 and MPL; or with QS21.

35

b) Groups of 5 Balb/c mice were vaccinated in the footpad with the different  
 vaccines (NS1-ICP27/NS1-ICP27 MPL + QS21/NS1-ICP27 SB26 = MPL and QS21/  
 adjuvant alone). One dose contained 10 µg NS1-ICP27, 10 µg MPL and 10µg QS21.

- 15 -

Two vaccinations were given at days 0 and 7. Mice were challenged at day 14 with  $5.2 \times 10^3$  TCID<sub>50</sub> of HSV2 strain MS. The appearance of zosteriform lesions and deaths were recorded until day 14 post challenge.

- 5 ICP27 of HSV2 was expressed in E coli as a fusion protein with NS1 fragment of influenza virus. The protective efficacy of the purified recombinant protein was evaluated in the murine zosteriform model, in combination with MPL QS21 formulations. Balb/c mice given two vaccinations with NS1-ICP27 combined either with MPL + QS21 or with an oil in water emulsion (SB26) + MPL and QS21 were
- 10 completely protected against disease (no zosteriform lesions) and death following HSV2 wild type challenge. In contrast, protection was not observed in the mice vaccinated either with NS1-ICP27 alone or with NS1-ICP27 combined with SB26 without MPL and QS21.

**Table 1**  
**Vehicles two fold concentrated**

Emulsions SB	Tocopherol %	Squalene %	Tween 80 %	Span 85 %	Lecithin %	Size
26	5	5	0.4	0	0	500 nm 90-100% 800 nm 10-0%
26.1	5	5	0.4	0	0.1	500 nm
63	5	5	0.6	0	0	500 nm
64	5	5	0.8	0	0	500 nm
61	5	5	1	0	0	250-300 nm
62	5	5	2	0	0	180 nm
40	5	5	0.4	1	0	500 nm 80-100% 800 nm 20-0%
40.1	5	5	0.4	1	0.1	500 nm
65	5	5	0.4	1.5	0	500 nm
65	5	5	0.4	2	0	500 nm

Table 2  
HIV gp 120W61D / MOUSE IMMUNOGENICITY (94243) / BALB / C (F.P.)

GROUPS	IMMUNOGEN (dose)/FORMULATION	ELISA TITERS (7 days PH)	% IgG1	%IgG2a	% IgG2b
1	gp120 10µg	494	100	0	0
2	gp120 10µg + 3D-MPL 5µg	4164	54	15	32
3	gp120 10µg + QS21 5µg	21515	89	4	8
4	gp120 10µg + 3D-MPL + QS21	52749	22	60	18
5	gp120 10µg / SB26	12205	94	2	4
6	gp120 10µg / SB26 + 3D-MPL	87388	31	42	27
7	gp120 10µg / SB26 + QS21	51020	73	15	13
8	gp120 10µg / SB26 + 3D-MPL + QS21	178169	23	57	21
9	SB26 + 3D-MPL + QS21 / gp120 10µg	185704	22	60	19
11	gp120 10µg / SB62	10348	92	8	0
12	gp120 10µg / SB62 + 3D-MPL + QS21	21739	54	37	9
13	gp120 10µg / SB40	36320	90	7	4
14	gp120 10µg / SB40 + 3D-MPL	285219	31	44	25
15	gp120 10µg / SB40 + QS21	48953	78	15	7
16	gp120 10µg / SB40 + 3D-MPL + QS21	209217	14	67	18
17	gp120 10µg / SB61	<50	-	-	-
18	gp120 10µg / SB61 + 3D-MPL	77515	31	50	19
19	gp120 10µg / SB61 + QS21	40737	74	13	13
20	gp120 10µg / SB61 + 3D-MPL + QS21	59673	29	57	14
21	gp120 5µg / SB26	25089	99	0	1
22	gp120 5µg / SB26 + 3D-MPL + QS21	242736	18	61	21

ELISA titers to gp 120 W61D: geomean of 5 individual titers, calculated by LINEST

- 18 -

Table 3  
3D-MPL based formulations: HIV project Monkey studies

Read-Out	GMT ELisa W61 D		GMT Neut. MN		DTH in vivo	CMI in vitro		
	P11	P111	P11	P111		LP	IL-2	$\gamma$ IFN
gp120 (100 $\mu$ g)/ o/w + MPL + QS21	60523	93410	1:500	>1:3200		+	ND	+
gp120 (20 $\mu$ g)/ o/w + MPL + QS21	52026	50150	1:500	1:2400		+	ND	+
"Historical" gp120 (100 $\mu$ g)/ o/w + MPL in guinea pigs		20064						

**Claims**

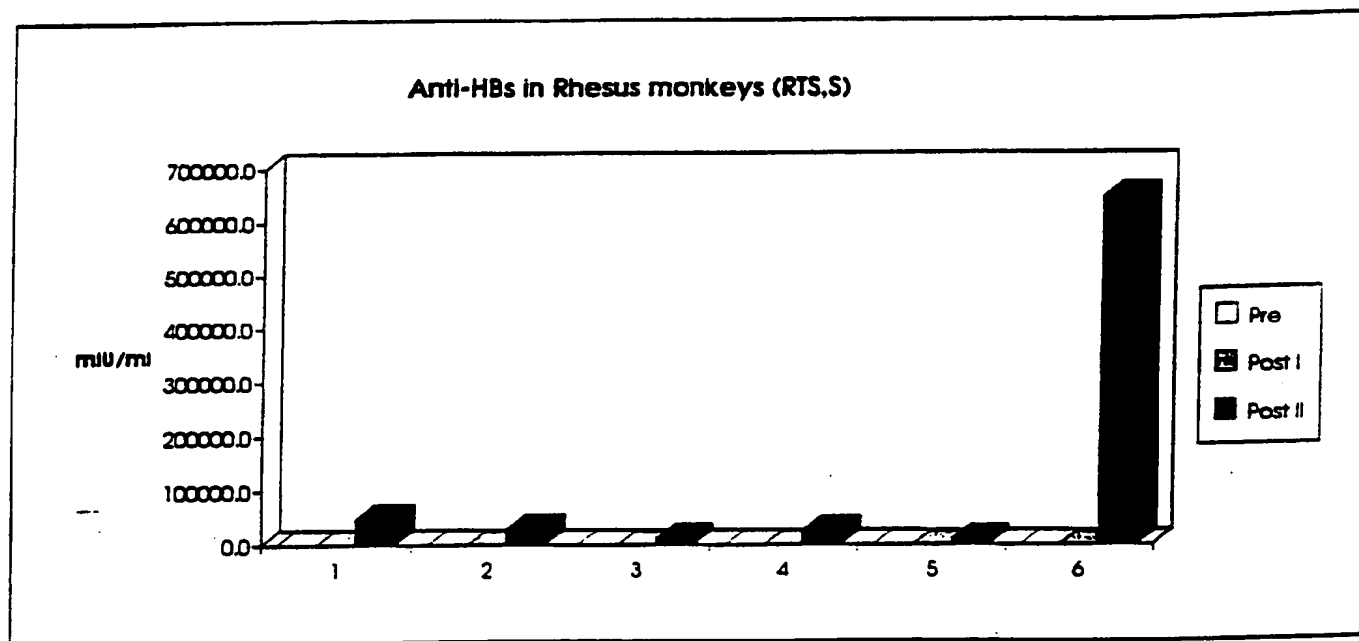
1. A vaccine composition comprising an antigen and/or antigenic composition, QS21, 3 De-O-acylated monophosphoryl lipid A (3D-MPL) and an oil in water emulsion wherein the oil in water emulsion has the following composition: a metabolisable oil, such as squalene, alpha tocopherol and tween 80.
2. A vaccine as claimed in claim 1 wherein the ratio of QS21:3D-MPL is from 1:10 to 10:1.
3. A vaccine composition as claimed in claim 1 or 2 capable of invoking a cytolytic T cell response in a mammal to the antigen or antigenic composition.
4. A vaccine composition as claimed in any of claims 1 to 3 capable of stimulating interferon  $\gamma$  production.
5. A vaccine composition as claimed in any of claims 1 to 4 wherein the ratio of QS21:3D-MPL is from 1:1 to 1:2.5.
6. A vaccine composition as claimed herein comprising an antigen or antigenic composition derived from any of Human Immunodeficiency Virus, Feline Immunodeficiency Virus, Herpes Simplex Virus type 1, Herpes Simplex virus type 2, Human cytomegalovirus, Hepatitis A,B,C or E, Respiratory Syncytial virus, human papilloma virus, Influenza virus, Salmonella, Neisseria, Borrelia, Chlamydia, Bordetella, Plasmodium or Toxoplasma.
7. A vaccine as claimed in any of claim 1 to 5 wherein the antigen is a tumour antigen.
8. Use of composition as defined in any of claims 1 to 5 for the manufacture of a vaccine for the prophylactic treatment of viral, bacterial, or parasitic infections.
9. Use of composition as defined in any of claims 1 to 5 for the manufacture of a vaccine for the immunotherapeutic treatment of viral, bacterial, parasitic infections or cancer.
10. A method of treating a mammal suffering from or susceptible to a pathogenic infection comprising the administration of a safe and effective amount of a composition according to any of claims 1 to 5.

11. A method of treating a mammal suffering from cancer comprising the administration of a safe and effective amount of a composition according to any of claims 1 to 5.

12. A process for making a vaccine composition according to claims 1 to 5 comprising admixing QS21, 3D-MPL and the oil in water emulsion as defined in claim 1 with an antigen or antigenic composition.

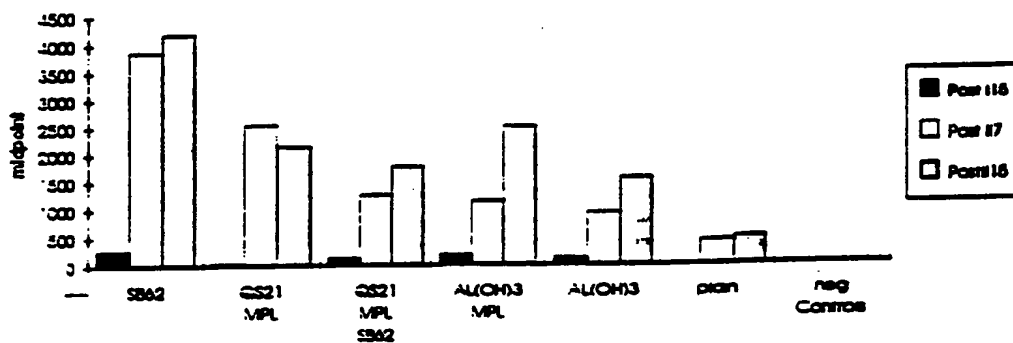
13. A vaccine composition comprising an antigen or antigenic composition in association with an oil in water emulsion which emulsion comprises: a metabolisable oil, alpha tocopherol, and tween 80.

1 / 4

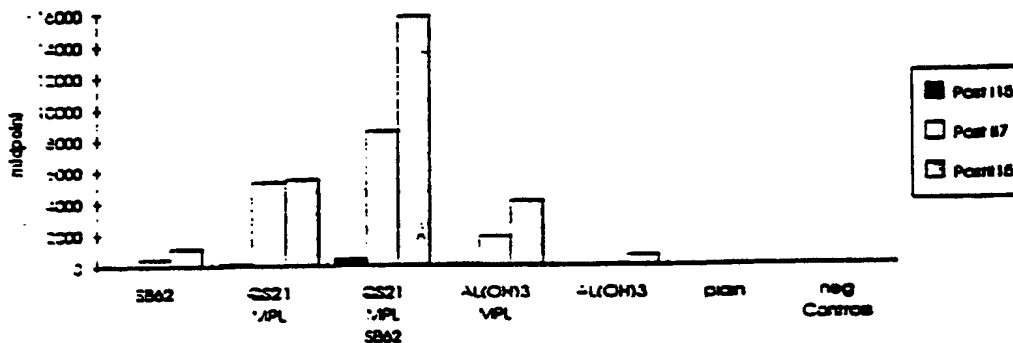
Figure 1

ANTIGEN HbsAg		IgG1			IgG2a			IgG2b		
GROUP	FORMULATION	Post 115	Post 117	Post 118	Post 115	Post 117	Post 118	Post 115	Post 117	Post 118
1	S362	275	3461	4171	44	479	1134	41	700	704
2	GS21 MPL	33	2533	2144	176	5301	5444	138	2235	1140
3	GS21 MPL S362	130	1244	1774	496	4551	15804	371	5107	3604
4	AL(OH)3 MPL	187	1138	2501	129	1832	4089	249	2021	1441
5	AL(OH)3	130	934	1562	13	128	684	265	192	981
6	plain	6	424	490	6	90	67	5	224	183
7	neg Control	5	5	5	5	5	5	5	5	5

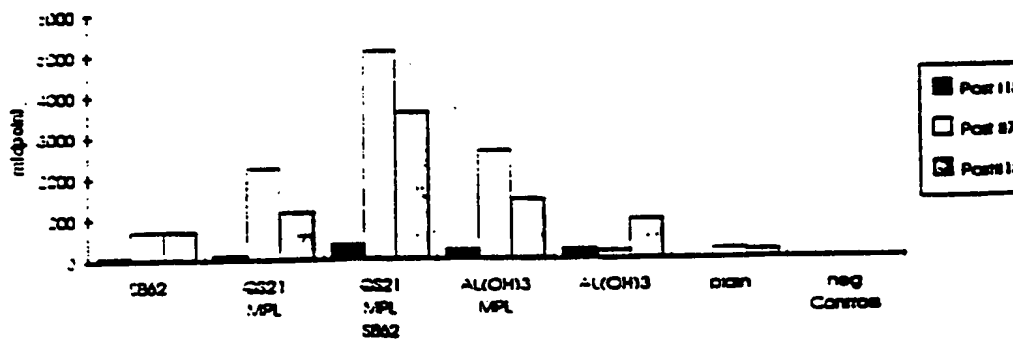
anti HbsAg IgG1 pool



anti HbsAg IgG2a pool



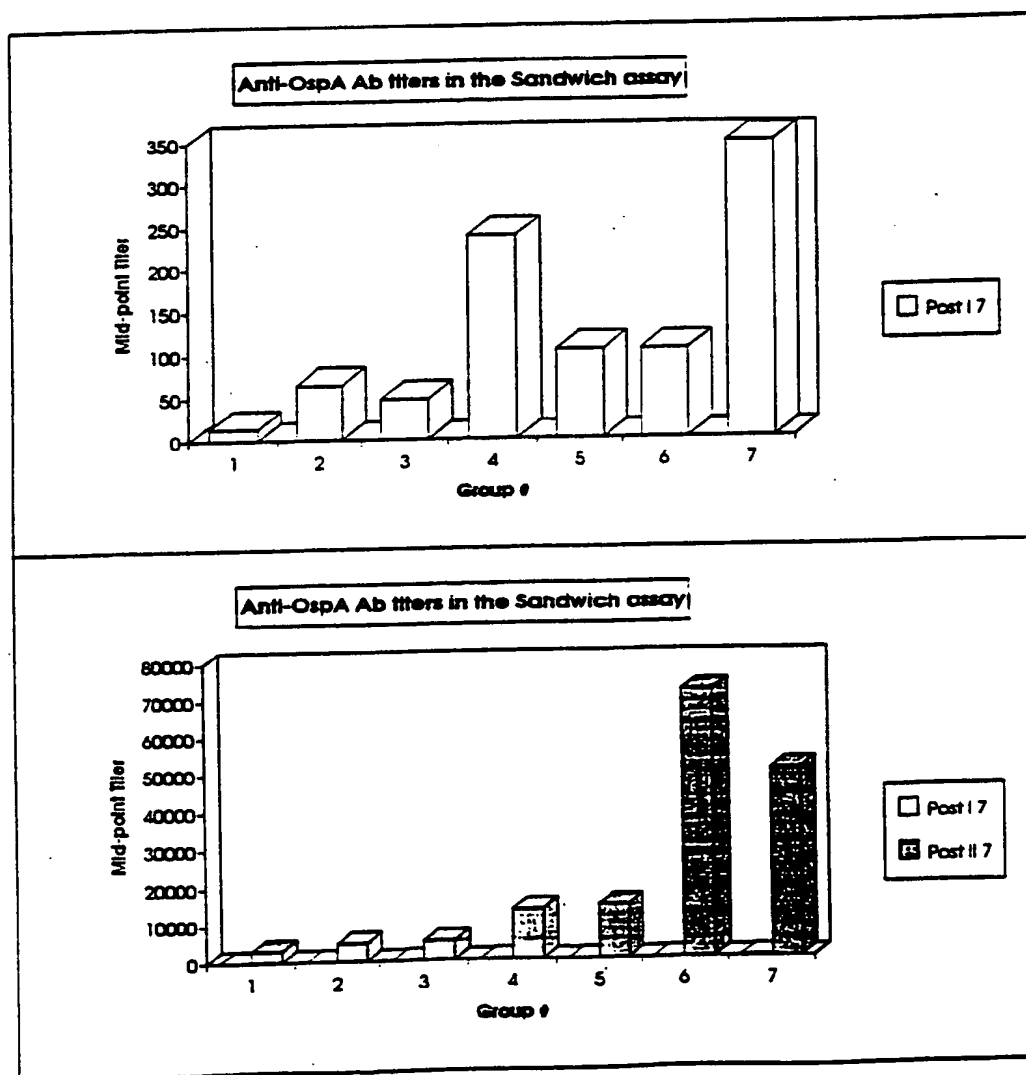
anti HbsAg IgG2b pool



3 / 4

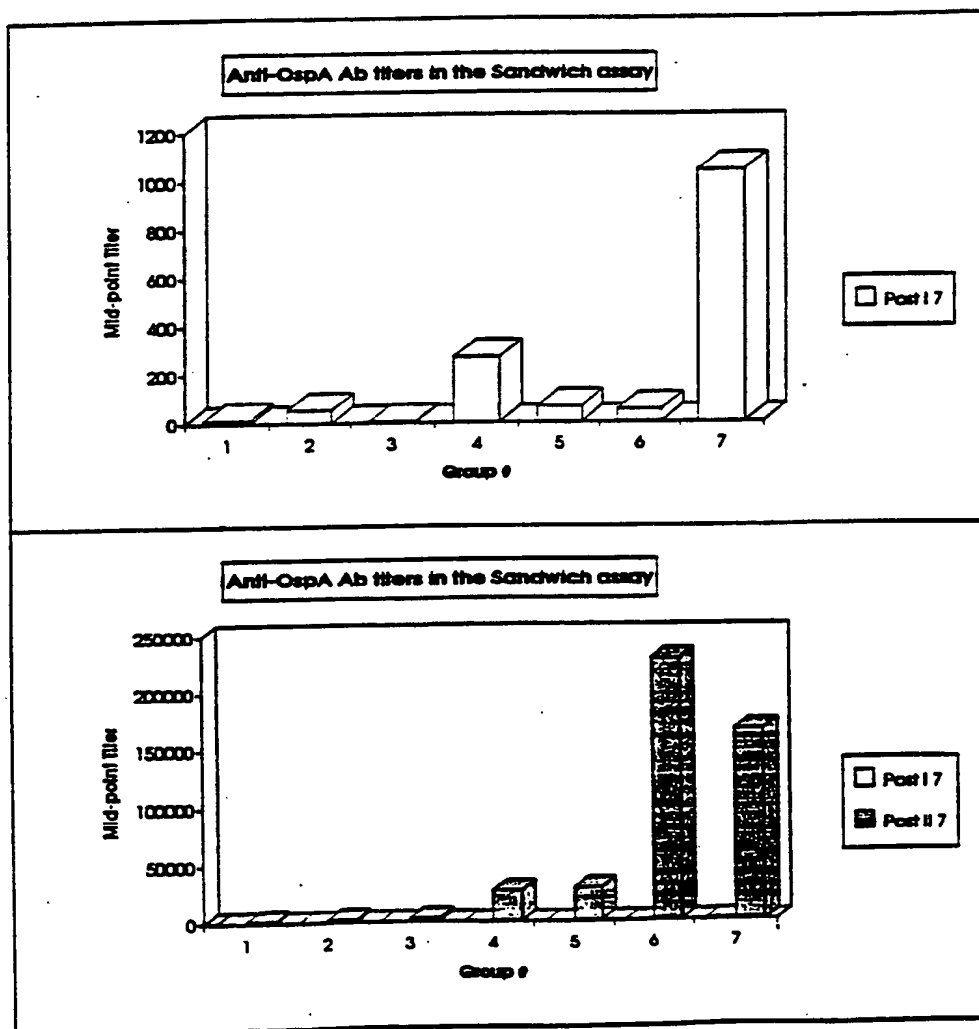
Anti-OspA Abs titers (igt) after immunization of Balb/C mice  
with different formulations of Lipoprotein OspA

Figure 3



Anti-OspA Abs titers (IgG2a) after immunization of Balb/C mice  
with different formulations of Lipoprotein OspA

Figure 4



# INTERNATIONAL SEARCH REPORT

Int. Application No

PCT/EP 94/04227

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 A61K39/39

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,92 16556 (SMITHKLINE BEECHAM BIOLOGICALS) 1 October 1992 cited in the application see the whole document ---	1-13
Y	EP,A,0 399 843 (CHIRON CORPORATION) 28 November 1990 cited in the application see the whole document ---	1,6-13
Y	EP,A,0 382 271 (AKZO) 16 August 1990 see the whole document ---	1,6-13
Y	WO,A,88 09336 (CAMBRIDGE BIOSCIENCE CORPORATION) 1 December 1988 cited in the application see the whole document ---	1,6-13
-/--		



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

\* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search

22 March 1995

Date of mailing of the international search report

30.03.95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Moreau, J

# INTERNATIONAL SEARCH REPORT

onal Application No

PCT/EP 94/04227

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	WO,A,94 00153 (SMITHKLINE BEECHAM BIOLOGICALS) 6 January 1994 see the whole document -----	1-13

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 94/04227

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 10-11 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 94/04227

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9216556	01-10-92	AP-A- 368	28-10-94
		AU-B- 654970	01-12-94
		AU-A- 8510991	28-04-92
		BR-A- 9107294	14-06-94
		CN-A- 1064891	30-09-92
		CZ-A- 9301957	18-05-94
		WO-A- 9206113	16-04-92
		EP-A- 0550485	14-07-93
		EP-A- 0644201	22-03-95
		HU-A- 67011	30-01-95
		JP-T- 6501151	10-02-94
EP-A-0399843	28-11-90	AT-T- 108327	15-07-94
		CA-A- 2017507	25-11-90
		DE-D- 69010574	18-08-94
		DE-T- 69010574	27-10-94
		ES-T- 2033626	16-10-94
		JP-T- 5508385	25-11-93
		WO-A- 9014837	13-12-90
EP-A-0382271	16-08-90	AT-T- 115862	15-01-95
		AU-B- 633043	21-01-93
		AU-A- 4897590	09-08-90
		CA-A- 2008856	04-08-90
		DE-D- 69015222	02-02-95
		JP-A- 2250835	08-10-90
WO-A-8809336	01-12-88	AT-T- 116993	15-01-95
		AU-B- 616670	07-11-91
		AU-A- 1934088	21-12-88
		CA-A- 1331443	16-08-94
		DE-D- 3852761	23-02-95
		EP-A- 0362279	11-04-90
		JP-T- 2504266	06-12-90
		US-A- 5057540	15-10-91
WO-A-9400153	06-01-94	AU-B- 4326393	24-01-94
		AU-B- 4326493	24-01-94
		CA-A- 2138996	06-01-94
		CA-A- 2138997	06-01-94

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

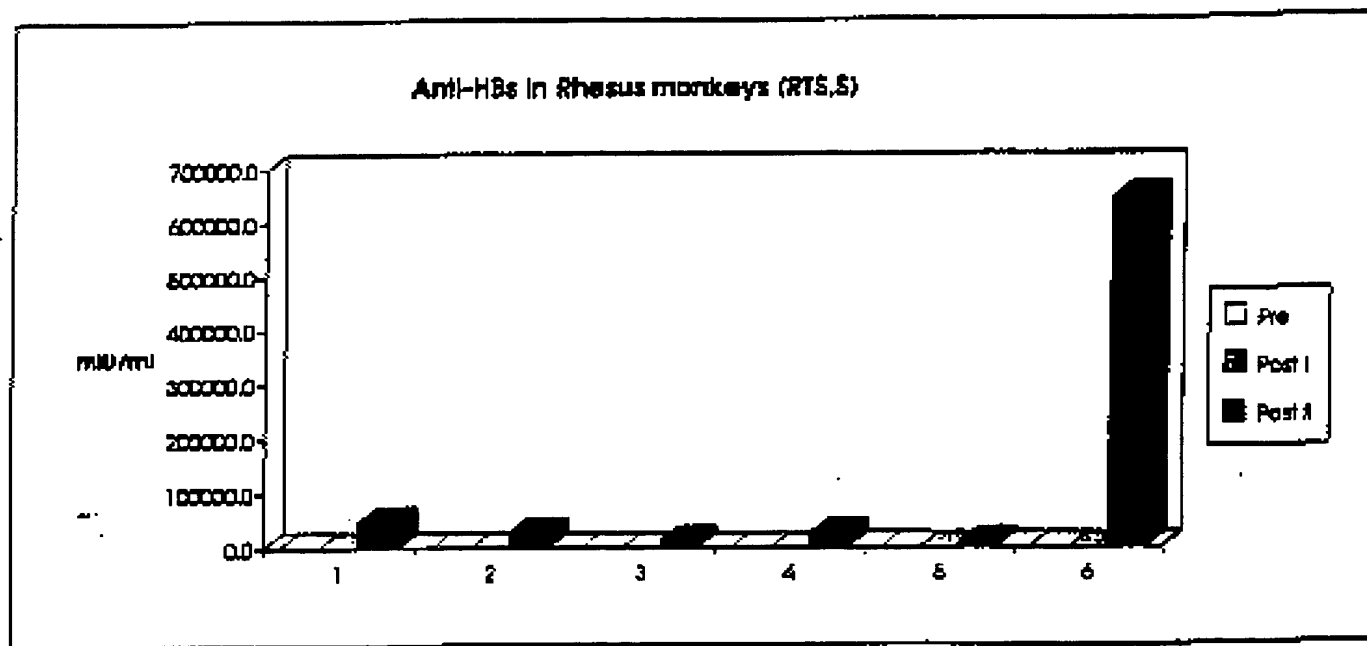
International application No

PCT/EP 94/04227

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9400153		CN-A- 1086142	04-05-94
		CN-A- 1092812	28-09-94
		WO-A- 9400575	06-01-94
		NO-A- 945003	23-12-94
		SI-A- 9300335	31-12-93
-----			

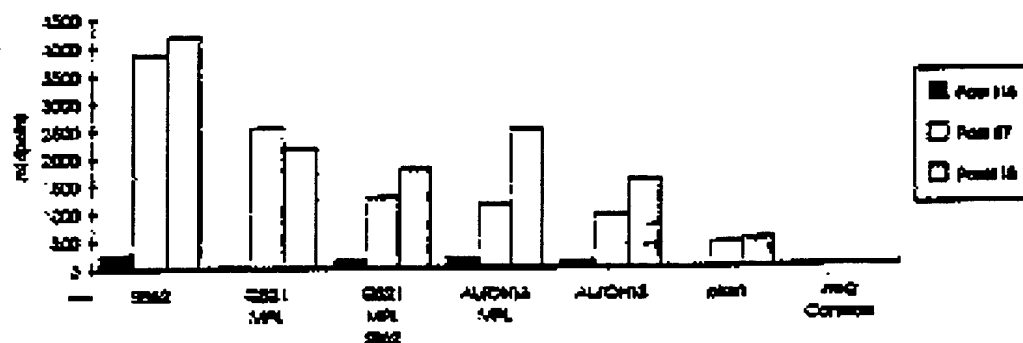


1/4

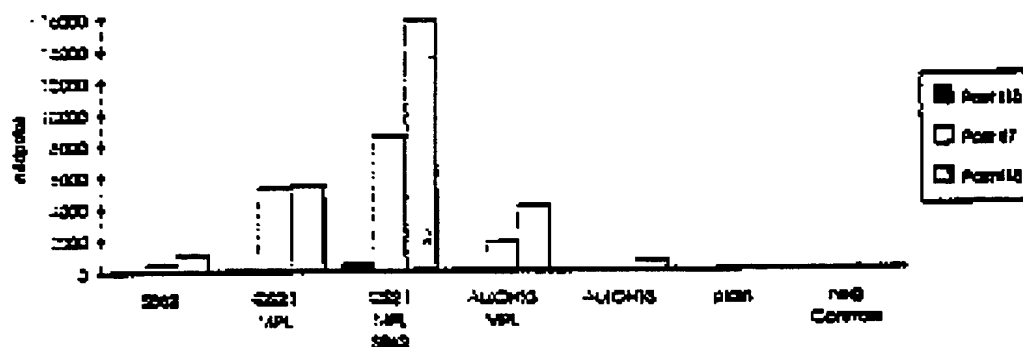
Figure 1

anti-HbsAg		IgG1			IgG2a			IgG2b		
GROUP	FORMULATION	Post 115	Post 127	Post 141	Post 115	Post 127	Post 141	Post 115	Post 127	Post 141
1	2302	275	3461	4171	46	479	1134	61	700	704
2	Q321 MPL	33	2533	2166	170	8307	3446	136	2236	1366
3	Q321 MPL 2302	120	1266	1776	496	6651	19666	371	8107	3606
4	ALONIS MPL	167	1136	2501	129	1862	4966	349	2621	1661
5	ALONIS	140	936	1666	12	126	666	266	191	661
6	place	6	426	496	6	96	46	6	266	166
7	neg Control	6	6	6	6	6	6	6	6	6

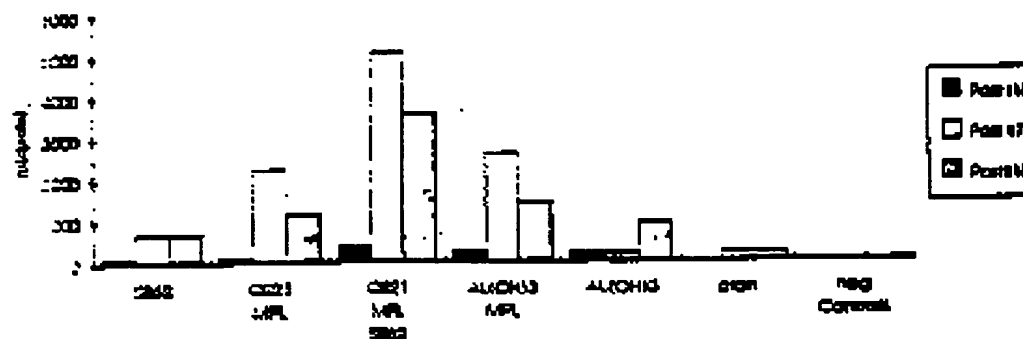
anti-HbsAg IgG1 pool



anti-HbsAg IgG2a pool



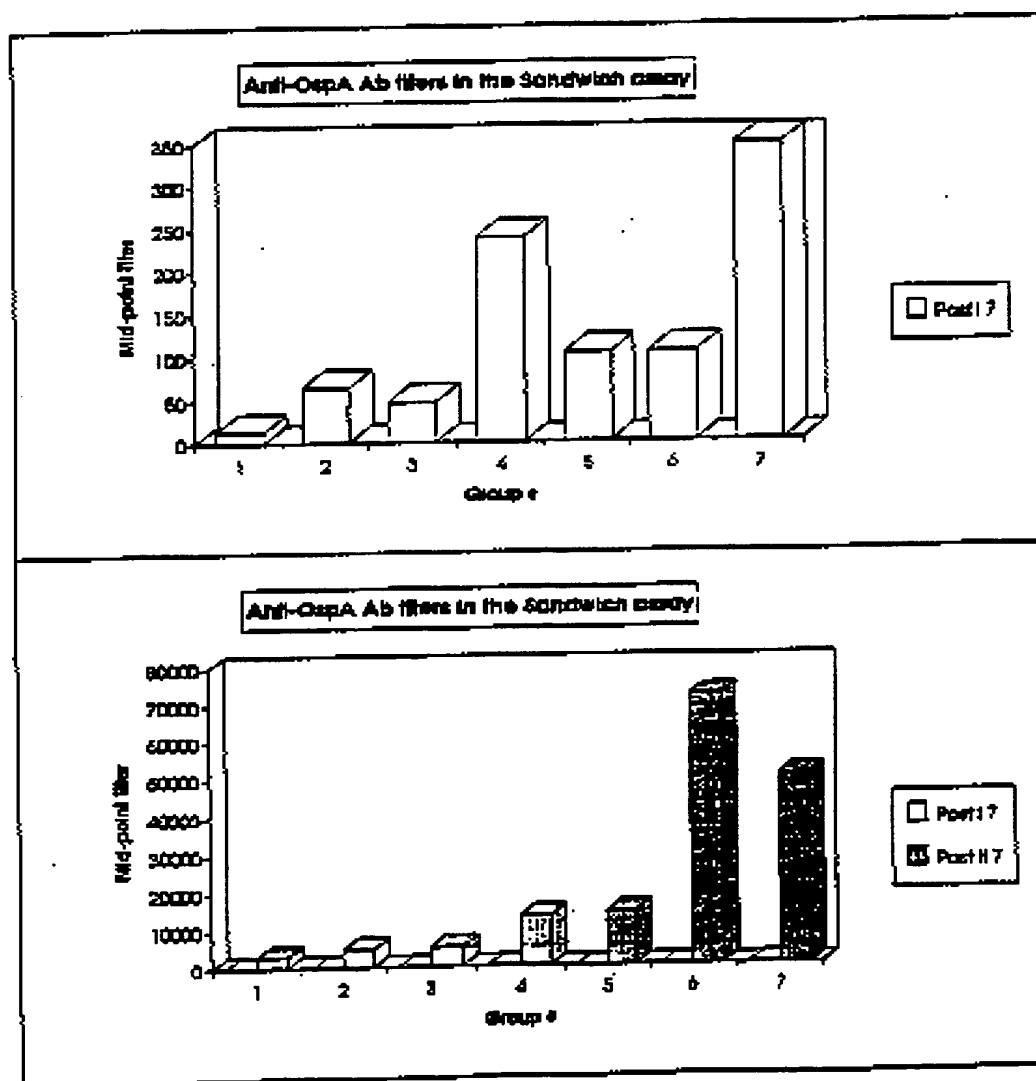
anti-HbsAg IgG2b pool



3/4

Anti-OspA Abs titres (igf) after immunization of Balb/C mice  
with different formulations of Lipoprotein OspA

Figure 3



4 / 4

Anti-OspA Abs titre (µg220) after immunization of B6B/C mice  
with different formulations of Unoprotein OspA

Figure 4

